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Efficacy and Safety of Frozen Blood for Transfusion in Trauma Patients

Martin A. Schreiber, Jerome A. Differding, Loic J. Fabricant, David A. Hampton, Laszlo Kiraly, Samantha J. Underwood, Dinh Le, Jennifer M. Watters

Oregon Health & Science University, Portland, OR

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NOTICE AND SIGNATURE PAGE

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ELIZABETH E. HEYD   DR. RODGER D. VANDERBEEK
Chief, Airmen Integration Research Support   Chair, Aeromedical Research Dept

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Martin A. Schreiber, Jerome A. Differding, Loic J. Fabricant, David A. Hampton, Laszlo Kiraly, Samantha J. Underwood, Dinh Le, Jennifer M. Watters

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14. ABSTRACT
Standard liquid red blood cells (SLRBCs) are approved for use up to 42 days. During this period, a known storage lesion occurs resulting in increased complications and death. We have previously shown that transfusion of cryopreserved deglycerolized RBCs (CDRBCs), which can be stored for 10 years, results in increased tissue oxygenation compared to SLRBCs. This study was performed to compare the biochemical properties and effects of CDRBCs to SLRBCs. Our hypothesis is that CDRBCs would have a similar profile with respect to the storage lesion compared to SLRBCs. Fifty-seven stable trauma patients who required one or two units of blood were randomized to receive SLRBCs or CDRBCs. Laboratory assays were performed on the donated blood and the patients prior to transfusion and on the patients 12 hours after transfusion. The median age of blood was 14 days in the SLRBC group and 21 months in the CDRBC group ($p<0.01$). Hematocrit increased similarly in both groups and remained similar throughout hospitalization. 2,3 diphosphoglycerate (2,3 DPG) was increased in patients who received CDRBCs and D-dimer levels increased in patients who received SLRBCs ($p<0.05$). Pro- and anti-inflammatory cytokines did not differ between groups after transfusion. Alpha-2 macroglobin, haptoglobin, C-reactive protein, and serum amyloid P were all higher in donated SLRBCs compared to CDRBCs ($p<0.01$). In conclusion, CDRBCs have superior characteristics with respect to the storage lesion compared to SLRBCs. Increases in 2,3 DPG are consistent with our prior findings of increased oxygenation in patients who receive CDRBCs.

15. SUBJECT TERMS
Transfusion, oxygenation, red blood cells, storage

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Introduction

A packed red blood cell (PRBC) transfusion is designed to treat anemia and increase end organ perfusion. Anemia in trauma patients is secondary to traumatic hemorrhage and the resulting coagulopathy. Subsequent resuscitation, surgical interventions, and serial blood draws result in further anemia. Red blood cell (RBC) production in these patients is often abnormal, and they are not able to recover from losses. As a consequence of anemia, many critically ill patients receive packed PRBC transfusions. The average ICU patient receives 5 units of PRBCs. There are several well accepted reasons to administer a PRBC transfusion including restoration and maintenance of intravascular volume, activation of platelet-driven hemostasis, restoration of tissue perfusion, and prevention of the development of organ failure and death. Its efficacy is based upon the red blood cells' ability to traverse a micro-capillary network and off-load oxygen. PRBC are stored using standard liquid preservation protocols. Donated blood mixed with citrate-phosphate-dextrose-adenine (CPDA) is centrifuged, the plasma is partially removed, and it is stored at 2-8 $^{\circ}$C. The US Food and Drug Administration (FDA) have approved liquid preserved RBCs (LPRBCs) for a 42-day shelf life, after which the blood must be destroyed. Each year 3% of donated blood is removed from circulation secondary to its expiration resulting in an estimated societal cost of $80 million. Blood transfusion is a critical therapeutic option. Unfortunately, there are seasonal or cyclical blood shortages that affect patient management. According to America's Blood Centers (ABC) STOPLIGHT blood availability monitoring program, on average, approximately 30 percent of national reporting sites had an available blood supply of only 2 days or less. Blood collected after national disasters usually takes 2 days to reach blood banks representing too long of a delay to be of immediate use to victims.

Banked donor blood is a mixture of cells with different ages and definite life spans. Standard preservation for banked blood is at a refrigerated temperature of 2-8 $^{\circ}$C. While awaiting usage, PRBCs undergo a collection of well-documented biochemical and structural changes known as the storage lesion. These changes adversely affect a cell's life span, oxygen off-loading capability, and ability to undergo the conformational changes necessary for navigating the capillary network. Depletion of nitric oxide (NO) impairs vaso-regulation, increasing intravascular resistance. Hemolysis and free hemoglobin scavenging of endothelial-derived NO further exacerbate this effect. Depletion of 2,3-diphosphoglycerate (2,3-DPG) impairs oxygen off-loading. Morphological alterations culminate in non-deformable spheroechinocytes which are unable to traverse capillary beds and impede circulation. This constellation of changes results in decreased tissue oxygenation (StO$_2$). Additionally, transfusions of LPRBC have been associated with prolonged hospitalizations, increased rates of infection, prolonged coagulation times, transfusion-related acute lung injury (TRALI), multi-organ failure (MOF), multiple organ dysfunction syndrome (MODS), post-operative complications in cardiac surgery and mortality. The association of these complications is more significant with prolonged LPRBC storage times. Currently blood banks distribute PRBCs from a queue; the oldest cells are the first to be transfused. Therefore critically-ill patients receive PRBCs which have assumed the maximum storage lesion. Attempts to avoid these time-dependent changes by shortening the 42-day storage period could potentially deplete national or regional reserves.

An alternative storage process, cryopreservation, pioneered by Dr. Audrey Smith in the 1950s, has the capacity to virtually eliminate the storage lesion. Cryopreservation, deep freezing to -80°C, utilizes glycerol as a buffer to prevent membrane injury and osmotic lysis secondary to extreme temperature changes. When required, the samples are thawed, deglycerolized, and transfused. The FDA requires donated blood to be glycerolized and frozen within 6 days and it is approved for a 10-year storage life. Per FDA mandate, once thawed the PRBC can be stored at 1-6 $^{\circ}$C for an additional 14 days. Recent literature has shown the storage lesion associated with cryopreserved cells is limited to the 6-day window prior to freezing. Arresting the storage lesion's evolution and extending the PRBC's shelf life could potentially eliminate transfusion-related complications seen with traditional storage methods.

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In 1966, units of cryopreserved PRBCs (CPRBC) were sent to the Naval Support Activity Hospital in Da Nang, Vietnam. During the South East Asian conflict, the FDA had approved a 21-day shelf life for LPRBCs. This truncated period resulted in destruction of nearly 50% of all donated blood. CPRBCs eliminated this deficit. Injured service members received CPRBCs without experiencing a transfusion related complication as compared with those receiving LPRBCs. For several decades following the Vietnam War, Massachusetts General Hospital, Chicago's Cook County Hospital and 30 other institutions throughout the United States developed and maintained robust cryopreserved blood programs. These efforts augmented their respective LPRBCs stores.

There is a growing body of evidence indicating that the duration of storage may affect the efficacy and safety of blood products. Ideally, a fresh product could be supplied to all recipients. However, in the absence of convincing data to support shortening the duration of refrigerated blood storage, the subsequent negative impact on blood bank inventory cannot be justified. During periods of high demand and low donation, typically during summer months, much of the national banked blood inventory is within days of expiring. Thus, reducing the storage period by as little as 1 week could significantly reduce the amount of available blood. Cryopreserved blood represents a possible method of solving this problem by avoiding the storage lesion without reducing the available blood supply. While there has been use of frozen RBCs in civilian and military practice, widespread application in the civilian setting has not yet occurred. Based on the theoretical advantages of cryopreserved blood, we performed a study to compare tissue oxygenation in patients receiving cryopreserved blood versus standard refrigerated blood. Clinical outcomes were assessed looking for evidence of gross safety issues with cryopreserved blood recognizing that the study was not adequately powered to make strong conclusions. The logistical benefits of cryopreserved blood have been well documented in the military and civilian settings. The biochemical benefits of this storage technique have not been rigorously studied and a prospective randomized trial comparing LPRBCs versus CPRBCs has never been performed. We hypothesized that CPRBCs have a superior biochemical profile as well as tissue oxygenation when compared to LPRBCs.

Body

Materials and Methods

This was a prospective, randomized, double blinded study. Institutional Review Board approval was obtained. Patients admitted to the Oregon Health & Science University trauma service with an Injury Severity Score (ISS) > 4 and the potential need for a blood transfusion were eligible. Patients who had received a massive transfusion within the last 3 months, a transfusion within the previous 24-hours, who were pregnant, or less than 15 years old were excluded. Patients needing emergent interventions were not included due to processing time. The thawing and deglycerolization process takes approximately two hours. In addition, patients with bilateral upper extremity injuries preventing the placement of a tissue oxygenation (StO2) monitor on the thenar eminence were excluded. Consent was obtained from the patient or designated medical representative. Enrolled patients were blindly randomized to three groups: LPRBCs ≤ 14 days old, LPRBCs ≥ 15 days old, or CPRBCs. There were no differences seen between the refrigerated groups during the analysis so they were combined to increase the power of the analysis between LPRBCs and CPRBCs. Patient demographic information [gender, age, mechanism of injury, ISS, Acute Physiology and Chronic Health Evaluation (APACHE) II score) and clinical outcomes (length of stay, acute respiratory distress syndrome (ARDS), acute renal failure (ARF), multi-organ failure (MOF), post transfusion fever, transfusion reaction, deep venous thrombosis (DVT), and mortality] were recorded. Organ failure data was collected to assess for gross safety issues. ARDS was defined as outlined by the American-European consensus conference. Acute Renal Failure was assessed using the RIFLE classification as outlined by the Acute Dialysis Qualitative Initiative Workgroup.

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All decisions to administer PRBCs were at the discretion of the primary treatment team. The general threshold for transfusion in our facility is hemoglobin less than 7 g/dl except for patients with severe head injury in whom the threshold is hemoglobin less than 8 g/dl.   Thawing and deglycerolization was performed at the time of randomization and units were transfused within 3 days of thawing. Post-thaw, cells were preserved in AS-3 solution at 4C. While FDA guidelines allow use of cryopreserved RBCs up to 14 days after thawing, all units were transfused within 72 hours to minimize confounding comparisons.

The method of cross matching varied depending on the patient's antibody screen results. Antiglobulin, a serological test, was performed by the Gel microtube methods when the patient's serum was found to be without antibodies. When no antibodies were present and there were no discrepancies in the ABO, RH type, an electronic cross match was performed. When no antibodies were present and the patient was otherwise ineligible for computer cross match, an immediate spin serological test was performed.

Once a transfusion order was placed for an enrolled patient, a near infrared spectroscopy (NIRS) device (Hutchinson Technology, Hutchinson, MN) was placed prior to RBC administration and tissue oxygenation (StO2) was measured continuously for at least 12 hours after completion of the transfusion. This device uses a thenar eminence sensor pad to assess tissue oxygenation (StO2). In patients with radial arterial lines or those with upper extremity injuries, the sensor was placed on the other hand. Near infrared spectroscopy is a previously validated monitoring system that measures tissue perfusion noninvasively using near infrared light to assess the ratio of oxygenated hemoglobin to total hemoglobin in the underlying tissue.

Changes in StO2 were defined as median differences in maximum or minimum tissue oxygenation during the transfusion and post-transfusion periods compared to baseline values. In order to avoid minute-to-minute variations which are characteristics of StO2 measurements and to better characterize the trends, an area under the curve (AUC) analysis was performed. The AUC during the baseline, transfusion, and post-transfusion periods was measured and compared within and between groups.

Before initiation and after completion of each transfusion, 10cc blood samples were obtained from the patients. After 300cc of the transfusion was completed, 12cc test samples were obtained from the LPRBC and CPRBC units. Final laboratory samples were obtained 12 hours after completion of the last transfusion. Based on data showing the association between blood cell transfusion and venous thromboembolism, thrombelastography (TEG) was performed on all samples to evaluate coagulation status. A complete blood count was also performed at baseline, post infusion and at 12 hours. All samples were additionally assessed for biochemical changes. Hemoglobin was analyzed by ELISA (Bethyl Laboratories, Montgomery, TX). Haptoglobin, serum amyloid P (SAP) and C-reative protein (CRP) were evaluated utilizing the Bio-Plex Pro Human Acute Phase 4-Plex Panel (Bio-Rad Laboratories Inc., Hercules, CA). SAP and CRP were studied due to their known anti-coagulation effects. Elevated levels of SAP, in the presence of heparin, can cause a hypocoagulable state. At supra-physiologic levels, SAP's anti-coagulation effects manifest in the absence of heparin. CRP, an SAP homologue, also retards coagulation. Finally, hemolysis during storage could be assessed through changes in haptoglobin, a free hemoglobin scavenger.

NO metabolites were measured with a Seivers Nitric Oxide Analyzer 280i (GE Analytical Instruments, Boulder, CO). 2,3-DPG levels were quantified with a commercially available kit (Roche Diagnostics, Indianapolis, IN). Cytokines (IL-2, IL-4, IL-6, IL-8, IL-10, GMCSF, TNFα and IFNγ) were measured using the Human Cytokine 8-Plex Assay (Bio-Rad Laboratories Inc., Hercules, CA). Coagulation data (Prothrombin Time (PT), Partial Thromboplastin Time (PTT), D-dimer, and fibrinogen) were measured using standard tests (Diagnostica Stago Inc, Parsippany, NJ). Thrombelastography (TEG) data were obtained using a TEG 5000 Thrombelastograph Hemostasis Analyzer System (Haemonetics, Braintree, MA). Non-citrated whole blood was placed in kaolin activated cups to do these assessments. TEG, a

measure of clot formation efficiency, is characterized by four parameters: R - time to onset of clotting, K - rate of clot formation, alpha angle - rate of fibrin cross-linking, and MA – maximum amplitude or clot strength.

All data were analyzed using SPSS version 19 (IBM, Armonk, NY). All continuous normally distributed data were analyzed using Student t-tests. All non-parametric data were analyzed using a Mann-Whitney U test. A multivariate analysis was performed to control for important clinical variables to include injury severity score, mechanism of injury, and gender.

Results

Fifty-seven trauma patients were enrolled and received PRBC transfusions. Thirty five received LPRBCs. Nineteen of the patients received blood stored for <15 days and sixteen for 15-42 days. Twenty-two were administered CPRBCs. Eleven (31%) of patients who received LPRBCs received 2 consecutive units compared to 9 (41%) of patients who received CPRBCs (p = 0.57). The median duration of transfusion was 135 (75, 209) minutes. The median pre-administration storage age of the standard units was 14 (10, 27) days compared to 21 (14, 27) months for the cryopreserved units. Post-thaw, all deglycerolized blood was administered within 3 days. The two groups were well matched for age, gender, trauma mechanism (blunt versus penetrating), ISS, and APACHE II scores (Table 1).

(N = 57)	Standard RBCs (n = 35)	Cryopreserved RBCs (n = 22)	p-value
Age in Years (IQR)	44 (30,54)	50 (26,66)	0.56
Gender (Male / Female)	23 / 12	16 / 6	0.58
Mechanism (Blunt / Penetrating)	34 / 1	19 / 3	0.29
ISS (IQR)	26 (13,33)	18 (10,27)	0.18
APACHE II (Mean $\pm$ SD)	11 $\pm$ 5	11 $\pm$ 5	0.88
Units Transfused	3.1 $\pm$ 2.4	3.6 $\pm$ 2.9	0.80

Table 1. Demographic information for 35 patients receiving liquid preserved blood and 22 patients receiving cryopreserved blood.

While this study was not powered to demonstrate statistically significant differences in clinical outcomes, additional data were also assessed to screen for any major safety differences. No gross differences were noted between the two groups when comparing rates of organ failure, transfusion reactions, deep venous thrombosis (DVT), or median hospital length of stay (LOS) (Table 2).

	Standard RBCs (n = 34)	Cryopreserved RBCs (n = 22)	p-value
Respiratory Failure	32%	32%	0.97
Acute Renal Failure	9%	5%	0.54
Multiple Organ Failure	12%	9%	0.75
Post Transfusion Fever	3%	0%	0.42
Transfusion Reaction	0%	0%	*
DVT	15%	32%	0.13
Mortality	0%	0%	*
Hospital Length of Stay (IQR)	11 (7,16)	16 (9,27)	0.10

Table 2 – In-hospital complications for 34 patients receiving liquid preserved blood and 22 patients receiving cryopreserved blood.

There were no differences observed in TEG parameters (Table 3), with the exception of a slightly greater α-angle in the cryopreserved group following transfusion of the second RBC unit.

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	Standard RBCs	Cryopreserved RBCs	p-value
Baseline (R)	6.0 ± 1.7	5.9 ± 1.8	0.85
Post Transfusion Unit 1 (R)	6.6 ± 1.6	6.0 ± 1.2	0.32
Post Transfusion Unit 2 (R)	6.4 ± 1.4	5.9 ± 2.1	0.50
12 hours Post Transfusion (R)	6.3 ± 1.9	6.9 ± 2.4	0.44
Baseline (MA)	70.1 ± 8.6	73.1 ± 8.2	0.27
Post Transfusion Unit 1 (MA)	70.8 ± 7.0	69.8 ± 8.9	0.71
Post Transfusion Unit 2 (MA)	66.4 ± 7.6	70.5 ± 7.3	0.19
12 hours Post Transfusion (MA)	71.3 ± 7.9	72.8 ± 7.4	0.57
Baseline (α-angle)	70.6 (67.3,73.2)	73.1 (67.7,75.9)	0.19
Post Transfusion Unit 1 (α-angle)	68.1 (64.3,72.2)	70.7 (65.5,75.4)	0.24
Post Transfusion Unit 2 (α-angle)	66.7 (62.0,69.2)	71.7 (68.3,75.6)	0.01*
12 hours Post Transfusion (α-angle)	68.3 (63.5,72.7)	68.3 (63.8,74.9)	0.89

Table 3 – TEG coagulation parameters compared between the two groups. R-time = time to clot. MA = maximum clot strength. α-angle = fibrin crosslinking. * Statistically Significant. No differences denoted between time points of any variable within groups.

Hematocrit (HCT) was evaluated pre transfusion, immediately post transfusion, 12 hours post transfusion, and prior to discharge. No differences were noted in HCT change following transfusion between the groups (Figure 1).
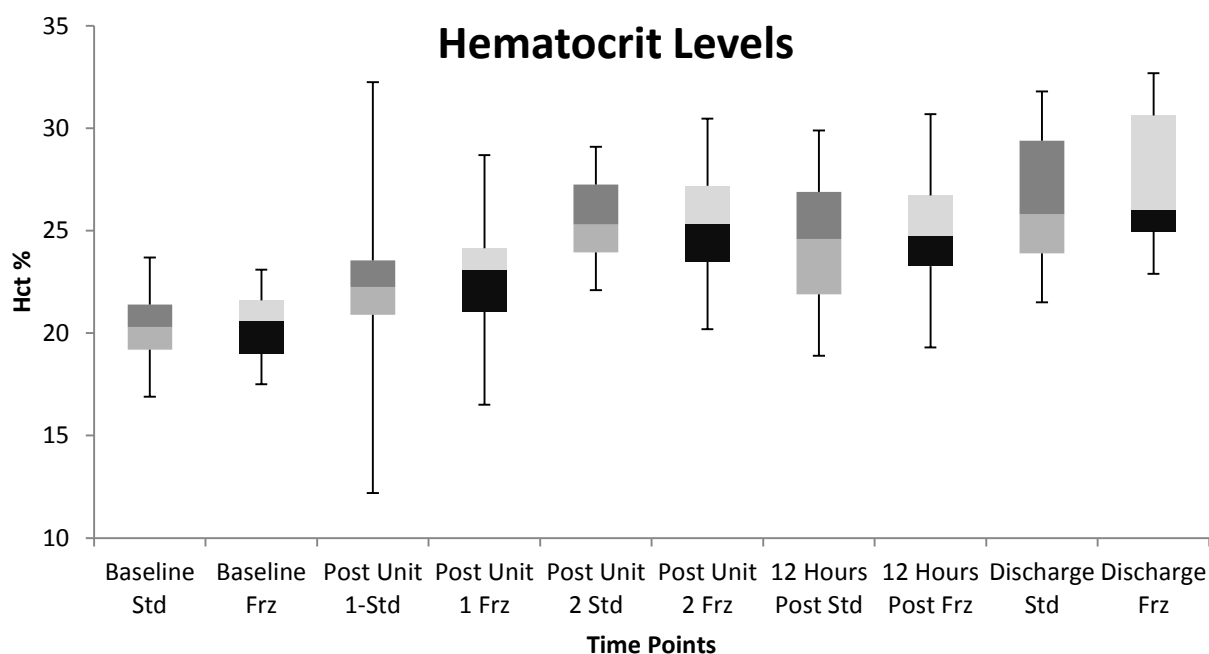
Figure 1. Mean hematocrit values at each time point, compared by group. Bars represent standard errors of mean.

Mean values for StO2 tracings were compared in patients receiving cryopreserved blood and standard RBCs (Figure 2). Tracings were excluded if recorded data was not continuous for the duration of transfusion through the 12 hour post-administration period. This resulted in 20 evaluable patients in the cryopreserved group and 30 in the standard group.
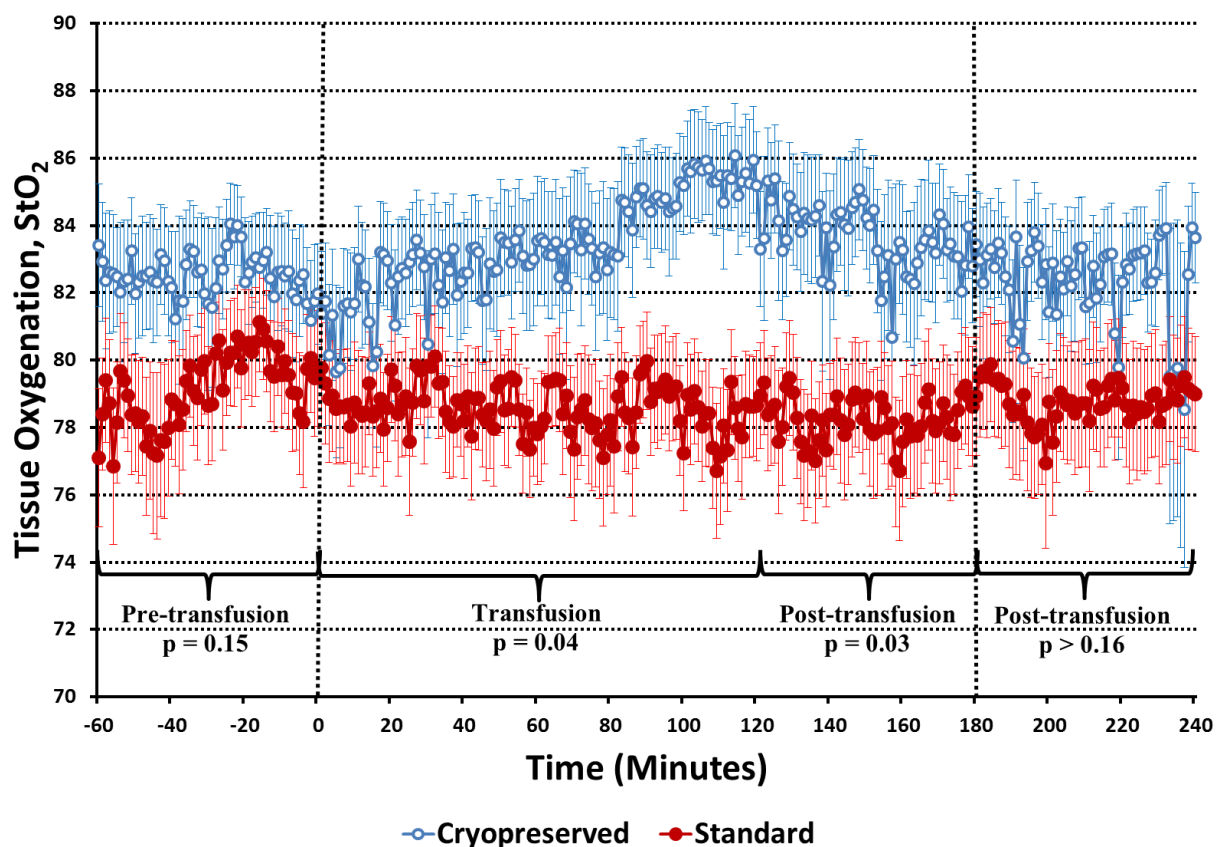
Figure 2 – Tissue oxygenation data from patients receiving liquid preserved RBCs (LPRBC) versus cryopreserved RBCs (CPRBCs)

A statistically significant difference was noted when comparing the AUC of mean StO_2 data for the two groups (p=0.03). This difference was observed from initiation of transfusion until the 180 minute time point. After that time, the differences were no longer statistically significant. Data were collected up to 12 hours after the completion of transfusion. After180 minutes, a trend of superiority continued, but slowly returned to baseline (Table 4).

Time	Type	n	Mean	Std. Deviation	p-value
AUC - 60 to 0	Standard	30	4825.8	523.8	
	Frozen	20	5034.4	435.9	0.15
AUC 0 to 120	Standard	30	9510.7	973.6	
	Frozen	20	10090.4	847.6	0.04
AUC 120 to 180	Standard	30	4783.4	538.9	
	Frozen	20	5102.6	450.8	0.03
AUC 180 to 240	Standard	30	4725.8	574.0	
	Frozen	20	4940.1	418.1	0.16
AUC 240 to 720	Standard	30	45605.4	8070.8	
	Frozen	20	47558.8	7468.7	0.39

Table 4 - Area under the curve of mean tissue oxygenation for cryopreserved blood versus standard blood.

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Median values of standard coagulation parameters of Prothrombin time (Pt), Activate Partial Thromboplastin Time (APTT), Fibrinogen (Fib) and Platelets (Plt) are represented in (Table 5). No differences were denoted within the groups at any time point comparison. Platelet function was significantly higher (p=0.01) in the cryopreserved group only after the transfusion of a second.

	Standard RBCs	Cryopreserved RBCs	p-value
Baseline (Pt)	14.8 (13.6, 16.0)	14.4 (13.3, 15.6)	0.37
Post Transfusion Unit 1 (Pt)	14.9 (13.8, 15.9)	14.0 (13.3, 15.9)	0.42
Post Transfusion Unit 2 (Pt)	15.1 (13.5, 16.7)	14.2 (12.7, 15.0)	0.15
12 Hours Post Transfusion(Pt)	14.2 (13.4, 15.2)	14.0 (12.7, 16.1)	0.87
Baseline (PTT)	41.1 (34.9, 46.2)	34.6 (31.0, 46.8)	0.29
Post Transfusion Unit 1 (PTT)	38.9 (33.7, 45.0)	33.7 (31.6, 49.6)	0.29
Post Transfusion Unit 2(PTT)	42.1 (33.9, 45.0)	35.1 (29.0, 45.5)	0.49
12 Hours Post Transfusion (PTT)	37.1 (32.1, 41.4)	36.4 (33.9, 49.8)	0.43
Baseline (Fib)	459.0 (398.0, 626.0)	476.0 (392.0, 667.0)	0.82
Post Transfusion Unit 1 (Fib)	428.0 (334.0, 667.0)	443.0 (312.5, 666.5)	0.89
Post Transfusion Unit 2 (Fib)	418.0 (327.8, 629.0)	530.0 (381.8, 725.6)	0.42
12 Hours Post Transfusion (Fib)	476.0 (392.0, 667.0)	504.0 (392.0, 667.0)	0.87
Baseline (Plt)	149.0 (112.0, 184.0)	183.0 (121.5, 264.3)	0.21
Post Transfusion Unit 1 (Plt)	143.0 (104.5, 169.5)	180.0 (140.5, 313.8)	0.08
Post Transfusion Unit 2 (Plt)	125.0 (96.5, 155.0)	205.5 (140.0, 275.8)	0.01*
12 Hours Post Transfusion (Plt)	137.0 (103.0, 195.0)	183.5 (134.3, 276.0)	0.12

Table 5 - Standard Coagulation Values Standard coagulation parameters of Prothrombin time (Pt), Activate Partial Thromboplastin Time (APTT), Fibrinogen (Fib) and Platelets (Plt) between the two groups. *Plt significantly greater in cryopreserved group.

Transfusion reactions were not seen in either group. Post-transfusion fever occurred in 1 patient in the LPRBC group. CPRBCs were stored for a significantly longer duration of time compared to the LPRBCs, 84 weeks (IQR: 56,108) versus 2 weeks (IQR: 1.4, 3.9) (Table 6, $p < 0.01$). Haptoglobin, SAP, and CRP were all significantly elevated in the LPRBC units compared to CPRBC units (Table 6, $p < 0.01$).

	Standard (N = 34)	Cryopreserved (N =22)
Storage duration (wks)	2 (1.4, 3.9)	84 (56, 108)
Haptoglobin (ng/ml)	73 (31, 105)	10 (4, 32)
C-reactive protein (ng/ml)	5 (2, 10)	0.06 (0.02, 1.65)
Serum amyloid P (ng/ml)	34 (22, 54)	1.8 (0.6, 13.5)

Table 6 – Stored blood characteristics for liquid stored and cryopreserved blood

Regarding the patients' laboratory work, after the 2nd transfused unit, 2,3 DPG was significantly higher in patients who received CPRBCs compared to patients who received LPRBCs [LPRBC: 0.52 mmol (IQR: 0.43, 0.86), CPRBC: 0.94 mmol (IQR: 0.53, 1.18), p=0.04]. The significant difference persisted 12 hours after the last transfusion [LPRBC: 0.48 (IQR: 0.32, 0.58), CPRBC: 0.76 (0.47, 1.37), p=0.01]. A difference in NO was not appreciated at any time point. Twelve hours after the last transfusion, D-dimer was significantly higher in the LPRBC group [LPRBC: 5.0 μg/ml (IQR: 0.2, 10.2) versus CPRBC: 2.9 μg/ml (IQR: 2.1, 5.3), p<0.05].

There were no differences in the CPRBC group's inflammatory cytokines (IL-2, IL-6, IL-8, GMCSF, INFγ, or TNFα) or the anti-inflammatory cytokines (IL-4 and IL-10) at any time point when compared to pre-transfusion values (Table 6). The 12-hour LPRBC group's IL-8, and TNFα were significantly elevated compared to pre-transfusion values [IL-8: 26.5 ng/ml (IQR: 16.6, 39.0) versus 29.2 ng/ml (IQR: 23.9, 41.5), $p<0.05$; TNFα: 11.1 ng/ml (IQR: 6.2, 19.5) versus 14.8 ng/ml (IQR: 6.7, 28.7), $p<0.05$] (Figures 2a, b). No other LPRBC group cytokine changes were observed.
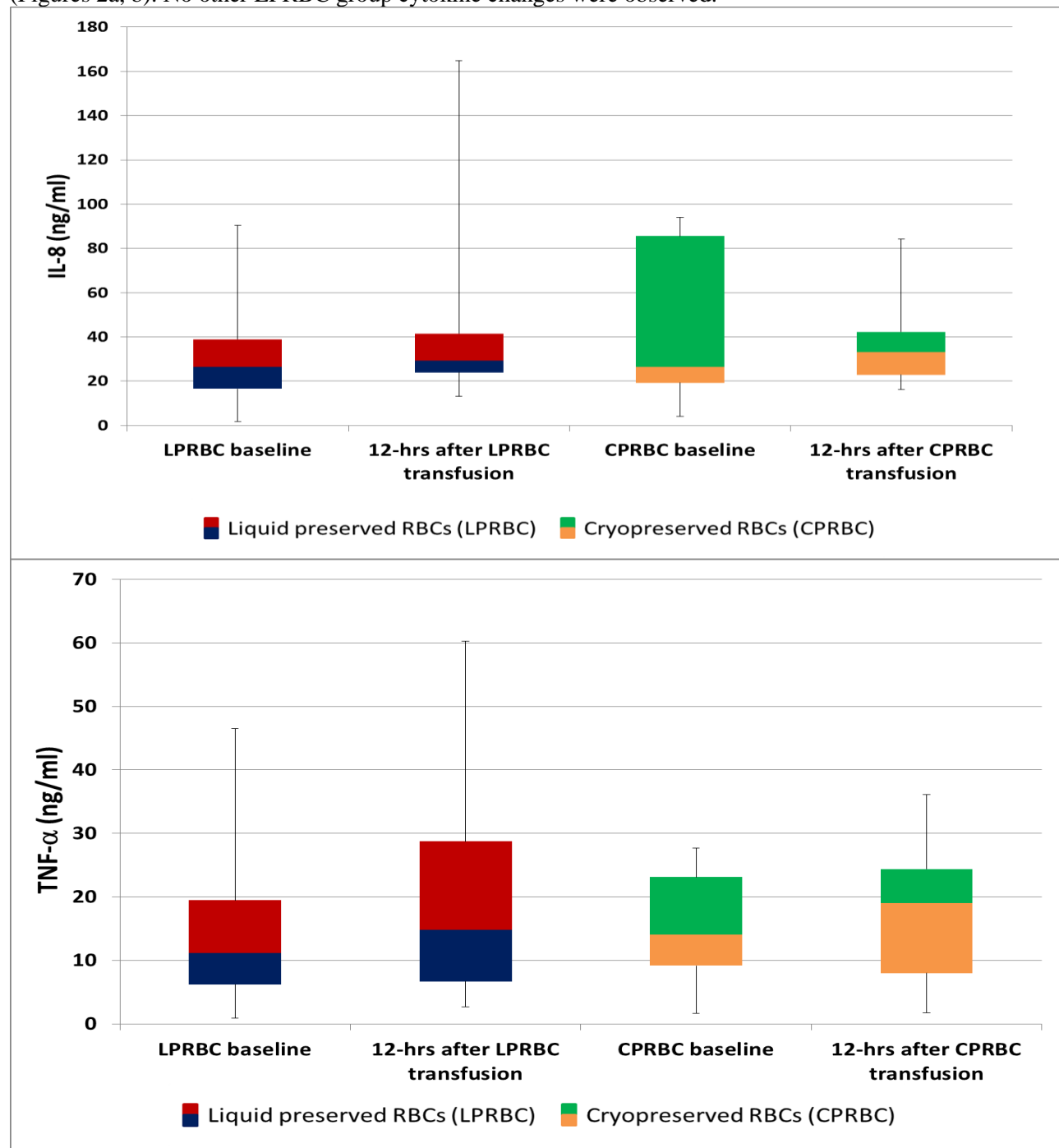

Figure 2a – IL-8 data from patients receiving liquid preserved RBCs (LPRBC) versus cryopreserved RBCs (CPRBC). IL-8 was significantly elevated 12 hours after transfusion in the LPRBC group ($p<0.05$). Figure 2b – TNF-□ data from patients receiving liquid preserved RBCs (LPRBC) versus cryopreserved RBCs (CPRBC). TNF-□ was significantly elevated 12 hours after transfusion in the LPRBC group ($p<0.05$).

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Key Research Accomplishments

1. Tissue Oxygenation significantly improved in cryopreserved blood until at least 180 minutes post transfusion.
2. Storage duration significantly longer in cryopreserved blood
3. Significantly improved results post transfusion in biochemical markers including
    a. Haptoglobin
    b. C-Reactive Protein
    c. Serum Amyloid
    d. 2,3 DPG
    e. D-Dimer
4. No difference in inflammatory markers in the CPRBC group
5. Significantly elevated inflammatory markers at 12 hour vs. Baseline in LPRBC

Reportable Outcomes

1. Presented at 2012 Annual ACS Oregon/Washington Meeting (June 2012)
2. Presented at Military Health System Research Symposium (August 2012)
3. Presented at Annual American Association for the Surgery of Trauma (September 2012)
4. Presented at and won 2012 Region X Committee on Trauma Competition (November 2012)
5. Will be Presented at Annual, National Committee on Trauma Meeting (2013)

Bibliography of Published Work from the Grant

1. Fabricant L et al., *Cryopreserved Deglycerolized Blood is Safe and Achieves Superior Tissue Oxygenation Compared to Refrigerated Red Blood Cells, A Prospective Randomized Study.* Journal of Trauma (in press).